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(54) Peptides with sulfate ester groups.

(57) Novel peptides having sulfate ester groups and containing 6 to 9 amino acids; possessing feeding inhibition properties and capable of stimulating the contraction of the gallbladder. Also methods of treating and preventing obesity in which these novel peptides or other specified peptides can be used.

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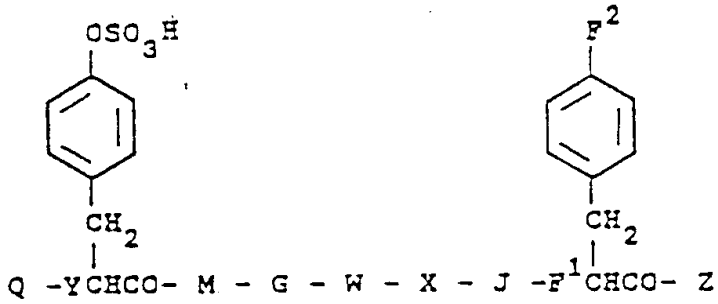
PEPTIDES WITH SULFATE ESTER GROUPS

BACKGROUND OF THE INVENTION

This invention concerns sulfate ester containing peptides possessing feeding inhibition properties and capable of stimulating the contraction of the gallbladder. These peptides have 6 to 9 amino acids. They all differ structurally, however, from two similarly sized peptides known to have feeding inhibition properties: CCK-8, which has the structure, Asp-Tyr(SO_3H)-Met-Gly-Try-Met-Asp-Phe-NH_2, and ceruletide, which has the structure, Glp-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2. The peptides of this invention are not found in nature but, rather, must be synthesized.

SUMMARY OF THE INVENTION

The compounds of the invention are peptides of the formula (1)



(1)

wherein
Q is H, H-Asp, H-βAsp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R^1R^2CHOCO,
Y is H, (S)-NH, (R)-R^3N, or (S)-R^3N,
M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, MeIle, or Lys,
G is Gly, DAla, Pro or Sar,
W is Trp, MeTrp or Nal,
X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, MeIle, Pro, or Lys,
J is Asp, DAsp, MeAsp, or Asn,
F^1 is (S)-NH, (S)-R^4N, or (R)-R^4N,
F^2 is H, Cl, I, Br, F, NO_2, NH_2, R^5, or OR^6,
Z is NH_2, NHR^7 or NR^7R^8,
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, and R^5 are lower alkyl,
R^6 is H or lower alkyl, and
R^7 and R^8 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that

(1) Q is desQ when Y is H,

(2) F^2 is not H if, in the same peptide, Q is H-Asp or Ac, Y is (S)-NH, M is either Met, MetO, Ahx or Leu, X is either Met, MetO, Ahx or Leu, G is Gly, DAla or Pro, W is Trp, J is Asp, F^1 is (S)-NH, and Z is NH_2,

(3) F^2 is not H if, in the same peptide, Q is H, H-βAsp or For, Y is (S)-NH, M is Met, Ahx or Leu, G is Gly, W is Trp, X is Met, Ahx or Leu, J is Asp, F^1 is (S)-NH, and Z is NH_2,

(4) F^2 is not H if, in the same peptide, Y is H, M is Met, X is Met, G is Gly, W is Trp, J is Asp, F^1 is (S)-NH, and Z is NH_2, and

(5) F^2 is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, G is Gly or DAla, W is Trp, J is Asp, F^1 is (S)-NH or (S)-R^4N, and Z is NH_2.

The invention is also a process of making the peptides of the invention.

The invention is also methods of treating obesity and preventing obesity, respectively; each such method comprising the administration, by either an intraperitoneal, intravenous, intramuscular, subcutaneous or intranasal route, to a mammal in need of such treatment, of a peptide of the formula (1) wherein

Q is H, H-Asp, H-βAsp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, H-Arg-Asp, Glp-Asp, Glp-Glu, Glp-Gln, Suc-Asp, Glt-Asp, Pht-Asp, R^9CO-Asp, Boc-Asp, Cbz-Asp, H-Abu, H-Aia, Boc, Cbz, or R^1R^2CHOCO,

Y is H, (S)-NH, (R)-R^3N, or (S)-R^3N,

M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, Melle Lys, Thr, Abu, Val, Mox, Gly, Phe, Tyr, or Trp,

G is Gly, DAla, Sar, DTrp, Pro, or βAla,

W is Trp, MeTrp, Nal, DTrp, Trp(Me), Trp(5-F), or Trp(6-F),

X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, Pro, Lys, DMet, Abu, or Mox,

J is Asp, DAsp, MeAsp, βAsp, or Asn,

F^1 is (S)-NH, (S)-R^4N, (R)-NH, or (R)-R^4N,

F^2 is H, Cl, I, Br, F, NO_2, NH_2, R^5, or OR^6,

Z is NH_2, NHR^7 or NR^7R^8,

R^1 and R^2 are independently H or lower alkyl,

R^3, R^4 and R^5 are lower alkyl,

R^6 is H or lower alkyl, and

R^7, R^8 and R^9 are lower alkyl,

and pharmaceutically acceptable salts thereof,

provided that

(1) Q is desQ when Y is H, and

(2) F^2 is not H if, in the same peptide, Q is H-Asp, Y is (S)-NH, M is Met, X is Met, G is Gly or Pro, W is Trp, J is Asp, F^1 is (S)-NH, and Z is NH_2.

In its first subgeneric aspect, the invention is defined as compounds of the invention with the exception that it is further limited so that

Q is H, H-Asp, H-βAsp, H-DAsp, For, Ac, Suc, desQ, or R^1R^2CHOCO;

Y is H, (S)-NH, or (S)-R^3N;

M is Met, MeMet, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, or Pro;

G is Gly or DAla;

W is Trp;

X is Met, MeMet, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, or Pro;

J is Asp;

F^1 is (S)-NH or (S)-R^4N;

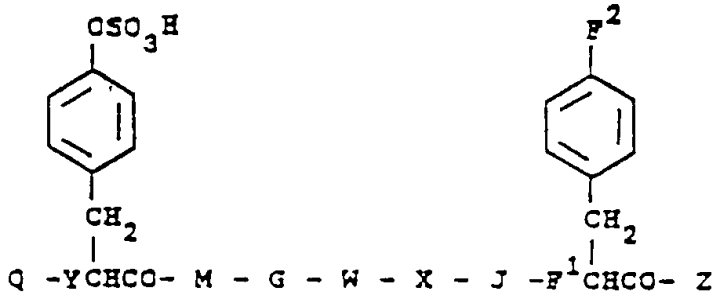
F^2 is H, Cl, NO_2, NH_2, R^5 or OR^6;

and Z is NH_2.

In a second subgeneric aspect, the invention is defined as in its first subgeneric aspect with the exception that it is further limited so that M is neither MeMet, MeAhx, MeLeu nor Melle, and X is neither MeMet, MeAhx, MeLeu, nor Melle.

Compounds with increased feeding inhibition activity over a 3-hour feeding period as compared to CCK-8 are:

A peptide of the formula



wherein

Q is H-βAsp, For, Suc, desQ, or R^1R^2CHOCO,

Y is H or (S)-NH,
M is Met, Ahx, Leu, or Ile,
G is Gly,
W is Trp,
X is Met, Ahx, Leu, or Ile,
J is Asp,
F^1 is (S)-NH or (S)-R^4N,
F^2 is H, NO_2, R^5, or OR^6,
Z is NH_2
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, R^5, and R^6 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that

(1) Q is desQ when Y is H,

(2) F^2 is not H if, in the same peptide, Q is H-βAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or Leu, X is either Met, Ahx, or Leu, and F^1 is (S)-NH,

(3) F^2 is not H if, in the same peptide, Y is H, M is Met, X is Met, and F^1 is (S)-NH,

(4) F^2 is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F^1 is (S)-R^4N.

In additional subgeneric aspects of the invention, the method of treating obesity or the method of preventing obesity is further limited to the administration of either the compounds of the invention or the compounds identified in the first or second generic aspects of the invention.

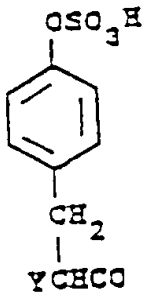
In a closely related invention, the compounds of the invention have Y as (R)-NH with the additional proviso that F^2 is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F^1 is (S)-NH, and Z is NH_2 so that compounds of this related invention are peptides of the formula (1) wherein
Q is H, H-Asp, H-βAsp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R^1R^2CHOCO,
Y is (R)-NH,
M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, MeIle, or Lys,
G is Gly, DAla, Pro or Sar,
W is Trp, MeTrp or Nal,
X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, MeIle, Pro, or Lys,
J is Asp, DAsp, MeAsp, or Asn,
F^1 is (S)-NH, (S)-R^4N, or (R)-R^4N,
F^2 is H, Cl, I, Br, F, NO_2, NH_2, R^5, or OR^6,
Z is NH_2, NHR^7, or NR^7R^8,
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, and R^5 are lower alkyl,
R^6 is H or lower alkyl, and
R^7 and R^8 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that
(1) F^2 is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F^1 is (S)-NH and Z is NH_2.

DETAILED DESCRIPTION

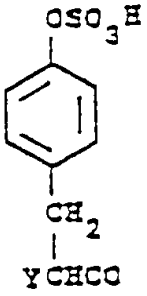
Definitions

"lower alkyl" contains 1 to 6 carbon atoms.

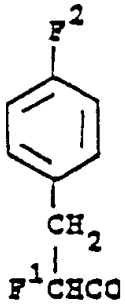
(R) and (S) refer to the absolute configurations, about the adjacent methine carbon. When Y is (S)-NH, then



is of the L-configuration and when Y is (R)-NH, then



is of the D-configuration. Similarly, when F^1 is (S)-NH or (R)-NH, then



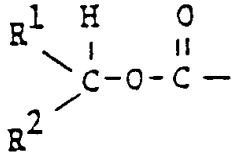
is of the L or D-configuration, respectively.

All optically active amino acids are of the L-configuration unless otherwise indicated.

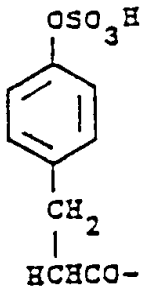
The H in H-Asp, H-βAsp, H-DAsp, H-MeAsp, H-Arg, H-Abu, and H-Ala stands for hydrogen.

DesQ, which arises when Y is H, means that there is no Q.

R^1R^2CHOCO is the formula



Hpp(SO_3H) is the formula



Each claim to a compound includes its pharmaceutically acceptable base addition salts. Base addition salts include those derived from both organic and inorganic bases, such as, for example, ammonia, sodium hydroxide, calcium hydroxide, barium hydroxide, tetraethylammonium hydroxide, ethylamine, diethylamine, triethylamine, and the like.

In an alternative representation to formula (1), used for purposes of brevity, peptides are also represented in accordance with conventional representation, for example,

H-Asp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2,

which stands for the compound of formula (1) in which Q is H-Asp, Y is (R)-NH, M is Met, G is Gly, W is Trp, X is Met, J is Asp, F^1 is (S)-NH, F^2 is H, and Z is NH_2.

When amino acids, peptides, protecting groups, active groups, etc. are represented by symbols in this specification and appended claims, usual symbols as defined by IUPAC and IUB or as used in the art are employed. Examples of symbols are given below.

Abu.....2-aminobutyric acid

Ac.....acetyl

Ahx.....2-aminohexanoic acid

Aib.....2-aminoisobutyric acid

Ala.....alanine

Arg.....arginine

Asn.....asparagine

Asp.....aspartic acid

βAsp..... beta-aspartic acid

Boc.....tert-butyloxycarbonyl

$BrCH_2$-Pam.....4-(bromomethyl)phenylacetamidomethyl

Cbz.....carbobenzoxy

Cys(Me).....S-methylcysteine

DAla.....D-alanine

DAhx.....D,2-aminohexanoic acid

DAsp.....D-aspartic acid

DMet.....D-methionine

DPhe.....D-phenylalanine

DPhe-NH_2.....D-phenylalanine amide

DTrp.....D-tryptophan

DTyr.....D-tyrosine

EtOCO.....ethyloxycarbonyl

EtPhe.....N-ethylphenylalanine

EtPhe-NH_2.....N-ethylphenylalanine-amide

Fmoc.....9-fluorenylmethyloxycarbonyl

For.....formyl

Gln.....glutamine

Glp.....pyroglutamyl

Glu.....glutamic acid

Glt.....$HOOC(CH_2)_3CO$-

Gly.....glycine

His.....histidine

Hpp.....3-(4-hydroxyphenyl)propionyl

Hpp(SO_3H).....3-(O-sulfo-4-oxyphenyl)propionyl

iBuOCO.....isobutyloxycarbonyl

Ile.....isoleucine

Leu.....leucine

Lys.....lysine

MeAhx.....N-methyl-2-aminohexanoic acid

MeAsp.....N-methylaspartic acid

MeLeu.....N-methylleucine

MeIle.....N-methylisoleucine

MeMet.....N-methylmethionine

MeOCO.....methyloxycarbonyl

MePhe.....N-methylphenylalanine

MePhe-NH_2.....N-methylphenylalanine amide

Met.....methionine

MetO.....methionine sulfoxide

MeTrp.....N-α-methyltryptophan

MeTyr.....N-methyltyrosine

MeTyr(Me).....N,O-dimethyltyrosine

MeTyr(Me)-NH_2.....N,O-dimethyltyrosine amide

Mox.....methoxinine

Nal.....3-(2-naphthyl)alanine

OBt.....1-benzotriazolyl ester

OCH_2-Pam.....4-(oxymethylphenyl)acetamidomethyl

OSu.....succinimidyloxy ester

OtBu.....tert-butyl ester

Phe.....phenylalanine

Phe-NH_2.....phenylalanine amide

Phe-NHEt.....phenylalanine ethylamide

Phe-NHMe.....phenylalanine methylamide

Phe-$N(Et)_2$.....phenylalanine diethylamide

Phe-$N(Me)_2$.....phenylalanine dimethylamide

Phe-OH.....phenylalanine acid

Phe(4-Cl).....3-(4-chlorophenyl)alanine

Phe(4-Cl)-NH_2.....3-(4-chlorophenyl)alanine amide

Phe(4-Me).....3-(4-methylphenyl)alanine

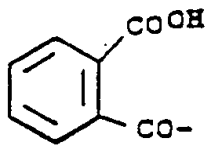
Phe(4-Me)-NH_2.....3-(4-methylphenyl)alanine amide

Phe(4-NO_2).....3-(4-nitrophenyl)alanine

Phe(4-NO_2)-NH_2.....3-(4-nitrophenyl)alanine amide

Phe(4-NH_2).....3-(4-aminophenyl)alanine

Phe(4-NH_2)-NH_2.....3-(4-aminophenyl)alanine amide

Pht..... 

Pro.....proline

PrOCO.....n-propyloxycarbonyl

resin.....polystyrene

Sar.....sarcosine

Ser.....serine

Suc.....$HOOC(CH_2)_2CO$-

tBu.....tert-butyl

Thr.....threonine

Trp.....tryptophan

Trp(5-F).....5-fluorotryptophan

Trp(6-F).....6-fluorotryptophan

Trp(Me).....1-methyltryptophan

Tyr.....tyrosine

Tyr-NH_2.....tyrosine amide

Tyr(Me).....O-methyltyrosine

Tyr(Me)-NH_2.....O-methyltyrosine amide

Tyr(SO_3H).....O-sulfotyrosine

Tyr(SO_3H)-NH_2.....O-sulfotyrosine amide

Val.....valine

Preferred Compounds

The preferred compounds of the invention are those that during the 0.5 hour feeding period inhibited feeding by 48-100% when administered at 30µg/kg in the test described under "Utility" below. (See Table 2 below.)

The most preferred compound, at the time of filing this continuation-in-part application, from the point of view of gallbladder contraction, is the compound Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2.

Particularly preferred compounds, at the time of filing this continuation-in-part application, from the point of feeding inhibition, were those that during the 0.5 hour feeding period inhibited feeding by 20-72% when administered at 0.3µg/kg in the test described under "Utility" below. They are:

H-DAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2
H-βAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
H-DAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
For-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
PrOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
EtOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
MeOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
H-βAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
H-Asp-Tyr(SO_3H)-Leu-Gly-Trp-Leu-Asp-Phe-NH_2
For-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2
Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2
Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
H-DAsp-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
H-Asp-DTyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

The compound, EtOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2, was the one that inhibited feeding by 72% at 0.3 µg/kg.

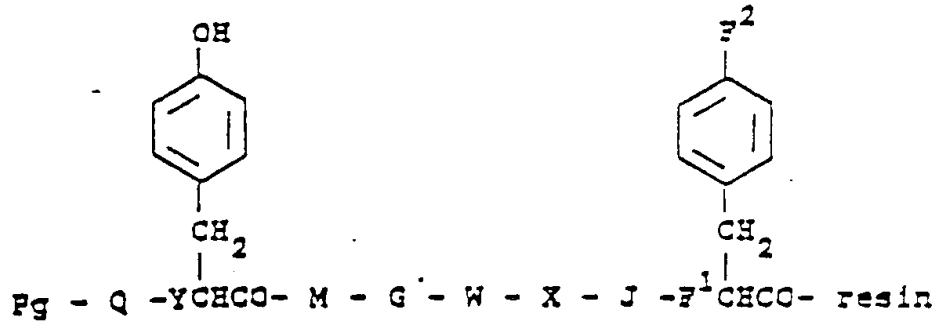
The most preferred compounds, at the time of filing this continuation-in-part application, from the point of view of feeding inhibition, were those that during the 3 hour feed period inhibited feeding by 50-90% when administered at 3 µg/kg in the test described under "Utility" below. They are:

iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2
For-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
PrOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2
Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2
Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2
For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2
Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

The compound, Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, was the one that inhibited feeding by 90% at 3 µg/kg during the 3 hour feeding period.

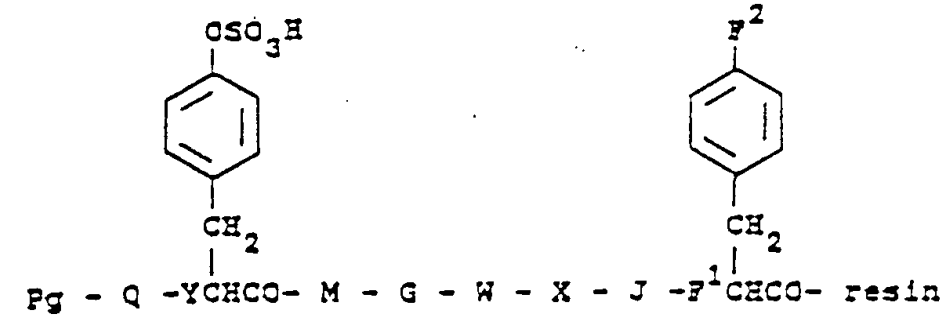
Preparation of Peptides

The novel sulfate ester peptides of this invention and the novel intermediates thereof may be prepared by methods well known to the art, for example, they may be prepared by combining individual amino acids on a solid phase resin on a step-by-step basis, or alternatively, by combining groups of amino acids on a solid phase resin to yield the desired peptidyl-resin intermediate. Such additions, as is known, are accomplished by protecting the amino group of the amino acid or group of amino acids by converting it to, for example, its tert-butyloxycarbonyl (Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc) derivative, and then activating the carboxylic group of such amino acid or group of amino acids by converting it, for example, to its 1-hydroxybenzotriazole (HOBt) or N-hydroxysuccinimide (HOSu) ester derivative. Such a protected-activated intermediate is then allowed to react with an amino acid-resin or peptidyl-resin with a free amino group, thus extending the peptide chain to provide the peptidyl-resin of formula 2, wherein Pg is a suitable protecting group, for example, Boc or Fmoc.



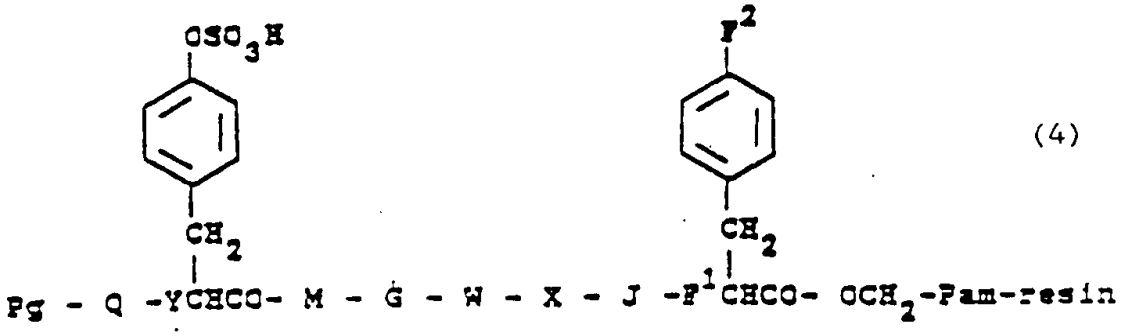
(2)

The phenolic OH group within formula (2) is converted to a sulfate ester by the use of a usual sulfating agent, such as sulfur trioxide pyridine complex. More specifically, the reaction is conducted, for example, by suspending a peptidyl-resin of formula 2 in dimethylformamide (DMF), pyridine or like solvent, and adding sulfur trioxide pyridine complex in about 10-40 molar excess to provide the sulfated peptidyl-resin of the formula 3.



(3)

Since the sulfate ester containing peptide end-products of this invention are C-terminal amides, the chemical link which connects the peptide chain to the resin must be such that its cleavage with suitable reagents readily provides amides. Due to the lability of the sulfate ester group to strong acids (for example, liquid hydrogen fluoride), the peptidyl-resin linkage may be cleavable with either weaker acids (for example, brief treatment with trifluoroacetic acid, TFA) and/or nucleophiles (for example, ammonia, amines, hydroxide, and alkoxides). Among the suitable resin derivatives may be mentioned oxymethyl-polystyrene, 4-(oxymethylphenyl)(CH_2)$_n$CO-aminomethyl-polystyrene (n = 0-3) and 4-(oxymethylphenyl)oxymethyl-polystyrene. Similarly substituted polyacrylamide resins are equally well suited as the above polystyrene based resins. For the purposes of this invention the 4-(oxymethylphenyl)CH_2CO-aminomethyl-polystyrene [herein referred to as 4-(oxymethylphenyl)acetamidomethylpolystyrene or OCH_2-Pam-resin] is best suited for the generation of peptide amides. Thus, this invention describes a process for the synthesis of sulfated peptidyl-OCH_2-Pam-resins of formula (4), wherein the resin is polystyrene (the term "polystyrene" includes copolymers with minor amounts, usually 1%, of unsaturated monomers such as divinylbenzene).



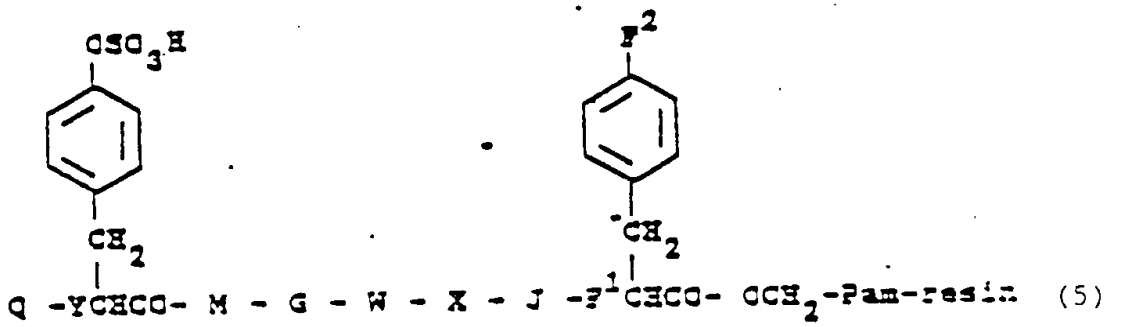
(4)

In forming peptide sequences of this invention, the amino functions may be protected by commonly used amino protecting groups such as Boc, Fmoc, (4-methoxybenzyl)oxycarbonyl, 2-nitrophenylsulfenyl, and so forth. The Boc and Fmoc protecting groups are preferred. The carboxyl and hydroxyl protecting group may be methyl, tert-butyl (tBu), benzyl, 4-methoxybenzyl and so forth. The tBu group is preferred.

The amino acid defined by the F^1 and F^2 groups of formula 4 may be attached to the OCH^2-Pam-resin in several ways. (a) For example, Boc protected-phenylalanine, wherein F^1 is (S)-NH and F^2 is H) may be reacted with a suitable 4-(bromomethyl)phenylacetate ester (for example, phenacyl ester) and processed further to provide Boc-Phe-(4-oxymethylphenyl)acetic acid which may be coupled to aminomethyl-polystyrene to provide Boc-Phe-(4-oxymethylphenyl)acetamidomethylpolystyrene (Boc-Phe-OCH_2-Pam-resin). (b) Alternatively, 4-(bromomethyl)phenylacetic acid may be coupled to aminomethylpolystyrene to provide 4-(bromomethyl)phenylacetamidomethylpolystyrene ($BrCH_2$-Pam-resin) which may be reacted with the cesium salt of Boc-Phe-OH to provide Boc-Phe-OCH_2-Pam-resin.

Among the suitable activating groups may be mentioned any combination of groups which causes the acid function of the amino acid to become more reactive, such as acid chlorides, mixed and symmetrical anhydrides, reaction product with carbodiimide (for example, dicyclohexylcarbodiimide, DCC), and active esters (for example, esters derived from HOBt, HOSu, 2-or 4-nitrophenol, and 2,4,5-trichlorophenol). The use of DCC and esters of HOBt and HOSu is particularly preferred from the standpoint of yield, lack of by-products, and consequent ease of purification.

The protecting groups are removed by known reactions such as treatment with dilute TFA (50% in dichloromethane, DCM) for Boc and/or tBu removal and treatment with dilute piperidine (20% in DMF) for Fmoc removal, to name a few, to provide the sulfated peptidyl-resin of the formula (5).



(5)

The sulfate ester containing peptides of formula 1 may be obtained by cleavage of the peptidyl-OCH_2-Pam-resin linkage of (5) with the appropriate reagent. The C-terminal sulfated peptide amides are derived, for example, by treatment of the sulfated peptidyl-resin of formula (5) with methanolic solutions of ammonia, alkylamines and dialkylamines.

An automatic peptide synthesizer was used for the solid phase synthesis of the sulfated peptide amides of this invention. The protocol of coupling onto aminomethyl-resin or peptidyl-OCH_2-Pam-resin (1 mmole of available nitrogen), deprotection, sulfation, cleavage, and product purification is set forth in Table 1.

Table 1. Protocol for solid phase synthesis of sulfated peptide amides (1 mmole scale). Each step volume is 50 ml unless otherwise indicated. All wash steps are repeated three times. Abbreviations: DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DIEA, N,N-diisopropylethylamine, DMF, dimethylfor-

mamide; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid.

Step	Reagent or Solvent	Purpose	Mix Time
1	DCM	Wash	1 min
2	Go to Step 3, 5, or 8	- - -	- - -
3	Add filtered, pre-activated (0°C, 1 hr) mixture of protected amino acid (or protected dipeptide, 3 mmole), HOBt (4.5 mmole), and DCC (3 mmole) in 1:4 DMF/DCM	Pre-activated DCC/ HOBt coupling	2-15 hr
4	Go to Step 10, 16, 21, or 26	- - -	- - -
5	Add protected amino acid (or protected dipeptide, 3 mmole) and HOBt (4.5 mmole) in 30 ml 1:2 DMF/DCM then DCC (3 mmole) in 20 ml DCM	In situ activated DCC/HOBt coupling	2-15 hr
6	2-Propanol	Wash	1 min
7	Go to Step 4	- - -	- - -
8	Add active ester or anhydride (3 mmole) in DCM, DMF, or mixture thereof	Non DCC/HOBt activated coupling	2-15 hr
9	Go to Step 4	- - -	- - -
10	DCM	Wash	1 min
11	Treat with 49:1:50 TFA/anisole/DCM	Boc and tBu removal	30 min
12	DCM	Wash	1 min
13	Treat with 1:19 DIEA/DCM	Neutralize	1 min
14	DCM	Wash	1 min
15	Go to Step 1, 16, 21, or 26	- - -	- - -
16	DMF	Wash	1 min
17	Treat with 1:4 piperidine/DMF	Fmoc removal	3 min
18	Treat with 1:4 piperidine/DMF	Fmoc removal	7 min
19	DMF	Wash	1 min
20	Go to Step 15	- - -	- - -
21	DMF	Wash	1 min
22	1:2 pyridine/DMF	Wash	1 min
23	Add sulfur trioxide pyridine complex (40 mmole) in 60 ml 1:2 pyridine/DMF	Sulfation	20-24 hr
24	DMF	Wash	1 min
25	Go to Step 4	- - -	- - -
26	Methanol	Wash	1 min
27	Ammonia saturated (-20°C) methanol or 20% methanolic amine (250 ml)	Resin cleavage	2-5 day
28	Methanol	Wash	1 min
29	Combine, concentrate filtrates from Steps 27-28	Isolation	- - -
30	Chromatograph residue on column(s) of Amberlite XAD-2 (Rohm and Haas, 2.5 x 60 cm, methanol gradient 0.1 M in ammonia), Trisacryl M DEAE (LKB Inc., 2.5 x 47 cm, ammonium bicarbonate gradient), and/or P-40 ODS-3 (Whatman, 4.8 x 50 cm, methanol gradient 0.2% in ammonium acetate)	Purification	- - -

The sulfate ester containing peptides of formula (1) thus prepared may be desalted and purified by the usual methods. For example, the product may be purified by ion-exchange chromatography with use of Trisacryl M DEAE, DEAE-cellulose or the like, partition chromatography with use of Sephadex LH-20, Sephadex G-25 or the like, reverse phase chromatography with use of Amberlite XAD-2, ODS-silica gel or the like, normal phase chromatography with use of silica gel or the like, or high-performance liquid

chromatography (HPLC).

Analogous procedures, wherein the reactions are carried out without the solid phase component (resin), are well known in the art and well suited to large scale production. [See, e.g., U.S. Patent 3,892,726.]

Utility

The peptides of this invention have the ability to inhibit feeding activity in mammals. As a result they have utility in the prevention and treatment of obesity. Feeding inhibition activity can be demonstrated in rats as follows:

Male Sprague-Dawley rats (weighing 300-350 g) are individually caged and maintained on a 12 hr light, dark cycle and trained for at least 14 days to feed during a three hr period of the dark cycle but not the 21 hours preceding that three hr period. The day of the study, rats are dosed intraperitoneally with saline (controls) or test compound (dissolved in saline; usually at a concentration of 0.3 to 300 micrograms of test compound per kg of rat weight). Food is introduced 10 minutes after administration of saline or test compound. Test compounds are deemed to be active if the test group consumes significantly less food than the saline controls during the feed period, which ends either 0.5 or 3 hr after presentation of food. The % feeding inhibition for the 1/2 hr and 3 hr feeding periods obtained by administering a dose of 30 μg/kg of test compound is given in Table 2 for CCK-8 and various compounds of the invention. For example, the % feeding inhibition obtained with CCK-8 (first line of Table 2) was 70% for the 1/2 hr feeding period and 25% for the 3-hour period.

TABLE 2

STRUCTURE	% FEEDING INHIBITION 0.5 hr-3 hr
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	70-25
H-DAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	99-40
iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	100-94
H-Asp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	74-24
H-Asp-Tyr(SO_3H)-DMet-Gly-Trp-Met-Asp-Phe-NH_2	17-IA
H-Asp-Tyr(SO_3H)-DAhx-Gly-Trp-Met-Asp-Phe-NH_2	14-17*
H-Asp-Tyr(SO_3H)-Met-Sar-Trp-Met-Asp-Phe-NH_2	55*-12*
H-Asp-Tyr(SO_3H)-Met-Gly-MeTrp-Met-Asp-Phe-NH_2	31-11
H-Asp-Tyr(SO_3H)-Met-Gly-Nal-Met-Asp-Phe-NH_2	52*-20*
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-DAsp-Phe-NH_2	32-15*
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asn-Phe-NH_2	43*-17*
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	70-24
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MeTyr(Me)-NH_2	65-17
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-NO_2)-NH_2	43-17
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-Cl)-NH_2	54-28*
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-NH_2)-NH_2	27-IA
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Tyr(Me)-NH_2	72*-10*
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NHMe	13*-IA
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NHEt	16-IA
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-N(Me)$_2$	64-27
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-N(Et)$_2$	18*-IA
H-βAsp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	47-33*
H-DAsp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	53-15
Suc-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	82-60

H-Asp-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2	85-20
H-Asp-Tyr(SO_3H)-Lys-Gly-Trp-Lys-Asp-Phe-NH_2	45*-IA
Hpp(SO_3H)-Met-DAla-Trp-Met-Asp-Phe-NH_2	59-16
Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2	100-97
H-βAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	100-78
H-DAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	100-72
For-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	100-96
iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	100-99
H-Asp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	85-35
Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	100-100
Hpp(SO_3H)-Met-DAla-Trp-Met-Asp-MePhe-NH_2	69-27
PrOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	90-74
EtOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	89-63
MeOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	100-91
H-βAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	91-21
H-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	50-30*
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	75-55
Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	99-75
H-Asp-Tyr(SO_3H)-Ahx-Gly-Trp-Met-Asp-Phe-NH_2	NT-NT
H-Asp-Tyr(SO_3H)-Met-Gly-DTrp-Met-Asp-Phe-NH_2	42*-IA
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Ahx-Asp-Phe-NH_2	82-23
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-DPhe-NH_2	46-14*
H-Asp-Tyr(SO_3H)-MetO-Gly-Trp-MetO-Asp-Phe-NH_2	15-IA
H-Asp-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2	82-27
H-Asp-Tyr(SO_3H)-Leu-Gly-Trp-Leu-Asp-Phe-NH_2	89-24
For-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	100-66

H-Asp-Tyr(SO_3H)-MetO-Gly-Trp-Met-Asp-Phe-NH_2	89-25
H-Asp-Tyr(SO_3H)-Met-DAla-Trp-Met-Asp-Phe-NH_2	16-18*
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-DMet-Asp-Phe-NH_2	30*-IA
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-MetO-Asp-Phe-NH_2	45-21*
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-Me)-NH_2	57-18
H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Tyr-NH_2	23*-IA
Hpp(SO_3H)-Met-Gly-Trp-Pro-Asp-Phe-NH_2	48-IA
Hpp(SO_3H)-Pro-Gly-Trp-Pro-Asp-Phe-NH_2	25-IA
Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2	100-98
iBuOCO-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2	100-100
Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2	94-96
iBuOCO-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2	100-100
Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2	100-99
Suc-Tyr(SO_3H)-Met-DAla-Trp-Met-Asp-Phe-NH_2	75-30
For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2	NT-NT
Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2	73-54
iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2	62-58
Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2	87-85
Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2	100-95
H-DAsp-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2	95-75
For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2	100-85
Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2	100-100
iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2	96-92
H-Asp-DTyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2	40-IA
Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2	100-97

IA means "inactive".

* - <10% inhibition at 30 µg/kg; number shown represents % inhibition at dose of 300 µg/kg.

NT means "not tested"

(END OF TABLE 2)

An appropriate procedure for administering a peptide designated here as suitable for intraperitoneal, intravenous, intramuscular, subcutaneous, or intranasal administration, to a mammal in need of either treatment for obesity or prevention of obesity, is at a dose of about 0.3 micrograms (µg) to 3 mg per kg of body weight per day, either as single dose or divided among two to four doses. The dosage may be varied, depending upon the requirements of the patient and the compound being employed.

The peptides of this invention have the ability to stimulate gallbladder contraction in mammals. Thus, they also find utility as diagnostic aids in X-ray examination of the gallbladder. The use of gallbladder contracting agents as diagnostic aids is a well established medical procedure.

EXAMPLES

The invention may be further illustrated by the following examples. Examples 1-18 illustrate the synthesis of intermediates. Exlamples 19-93 illustrate the synthesis of compounds that have been designated above as either a compound of the invention or a compound useful in the treatment and prevention of obesity. The examples are intended to illustrate the invention, not to limit it in any manner.

Peptide syntheses, unless otherwise stated, were initiated with 1 milliequivalent of aminomethyl resin, where the resin was 99:1 by weight styrene:divinylbenzene copolymer. Reactions were performed at room temperature unless otherwise stated.

Washing steps were performed three times with 50 ml of the specified solvent unless otherwise stated.

EXAMPLE 1

Isobutyl Succinimidyl Carbonate (iBuOCO-OSu)

To a solution of isobutyl chloroformate (26 ml, 200 mmole) in 600 ml of chloroform was added in portions the dicyclohexylamine (DCHA) salt of N-hydroxysuccinimide (HOSu, 49.28 g, 200 mmole). After stirring the resulting suspension overnight, the precipitated DCHA hydrochloride was filtered off and washed with chloroform. The concentrated filtrate (ca. 50 ml) and washings were diluted with 400 ml of ethyl acetate (EtOAc) and washed with 10% citric acid (4 x 100 ml), brine (2 x 100 ml), 10% sodium bicarbonate (3 x 100 ml), and brine (4 x 100 ml) and then dried (magnesium sulfate), filtered, and concentrated to ca. 100 ml. On diluting with ether and precipitating with hexane, 26.6 g (62% yield) of iBuOCO-OSu was obtained, mp 33-35°C.

EXAMPLE 2

Fmoc-DTyr-OH

D-Tyrosine (1.81 g) was dissolved in 20 ml of water and 30 ml of tetrahydrofuran (THF) with 10 ml of N sodium hydroxide. Solid 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 3.37 g) was added with rapid stirring. The suspension was adjusted to pH 7 with N sodium hydroxide and stirred overnight. Solid citric acid (3 g) was added followed by 60 ml of EtOAc. The EtOAc layer was collected, washed with 10% citric acid, brine, and dried (magnesium sulfate). Evaporation of the EtOAc solution gave a light tan syrup which was crystallized from dichloromethane (DCM) to give 3.9 g of Fmoc-DTyr-OH, mp 178-181°C.

EXAMPLE 3

Fmoc-Tyr-OH

Following the procedure of Example 2 but substituting L-tyrosine (9.06 g) for D-tyrosine, 18.4 g of Fmoc-Tyr-OH was obtained, mp 172-177°C.

EXAMPLE 4

Fmoc-MeTyr-OH

Following the procedure of Example 2 but substituting N-methyltyrosine (1.95 g) for D-tyrosine, 1.22 g of Fmoc-MeTyr-OH was obtained, mp 152-158°C.

EXAMPLE 5

Boc-MePhe-(4-oxymethylphenyl)acetic Acid

To a solution of Boc-MePhe-OH (27.93 g) and 4-(bromomethyl) phenylacetic acid phenacyl ester (33.32 g) in 1000 ml of acetonitrile was added potassium fluoride dihydrate (18.28 g). The suspension was stirred overnight, filtered and the filtrate evaporated to dryness. The residue, Boc-MePhe-(4-oxymethylphenyl)-acetic acid phenacyl ester, was dissolved in 85% acetic acid (1200 ml), treated with zinc dust (128 g), and stirred for 2-4 hrs. Concentration of the filtered reaction mixture to ca. 400 ml and dilution with ca. 3200 ml of water gave an oil which was dissolved in EtOAc and treated with DCHA to give 41.31 g of the DCHA salt of title compound, mp 120-122°C.

EXAMPLE 6

Boc-EtPhe-(4-oxymethylphenyl)acetic Acid

Following the procedure of Example 5 but substituting Boc-EtPhe-OH (7.33 g) for Boc-MePhe-OH, 5.69 g of the DCHA salt of Boc-EtPhe-(4-oxymethylphenyl)acetic acid was obtained, mp 137-141°C.

EXAMPLE 7

Boc-Phe(4-Cl)-(4-oxymethylphenyl)acetic Acid

Following the procedure of Example 5 but substituting Boc-Phe(4-Cl)-OH (2.5 g) for Boc-MePhe-OH, 3.44 g of the free base of Boc-Phe(4-Cl)-(4-oxymethylphenyl)acetic acid was obtained.

EXAMPLE 8

Boc-Tyr(Me)-(4-oxymethylphenyl)acetic Acid

Following the procedure of Example 5 but substituting Boc-Tyr(Me)-OH (2.5 g) for Boc-MePhe-OH, 1.83 g of the free base of Boc-Tyr(Me)-(4-oxymethylphenyl)acetic acid was obtained, mp 64-67°C.

EXAMPLE 9

Fmoc-Tyr(tBu)-(4-oxymethylphenyl)acetic Acid

Following the procedure of Example 5 but substituting Fmoc-Tyr(tBu)-OH (6.86 g) for Boc-MePhe-OH, 4.88 g of the free base of Fmoc-Tyr(tBu)-(4-oxymethylphenyl)acetic acid was obtained, mp 192-195°C.

EXAMPLE 10

Fmoc-Met-Asp(OtBu)-OH

Fmoc-Met-Osu was prepared, in situ, by the reaction of Fmoc-Met-OH (14.87 g), HOSu (5.52 g), and dicyclohexylcarbodiimide (DCC, 8.26 g) in THF (200 ml) at 0°C for 3.5 hrs. Precipitated dicyclohexylurea (DCU) was removed by filtration and the THF filtrate was added to a cold solution of H-Asp (OtBu)-OH in 220 ml of 10:1 water/THF to which had been added 40 ml of N sodium hydroxide. After stirring the reaction mixture at room temperature overnight, solid citric acid (20 g) was added along with EtOAc (600 ml). The EtOAc layer was separated, washed with 10% citric acid and brine, and dried (magnesium sulfate). Evaporation of the EtOAc solution gave a residue which was dissolved in 200 ml of EtOAc and treated with DCHA (7.84 ml) to precipitate 17.93 g of the DCHA salt of the desired product, mp 159-162°C.

EXAMPLE 11

H-Phe-OCH_2-Pam-resin

Boc-Phe-(4-oxymethylphenyl)acetic acid (0.83 g, 2 mmole), 1-hydroxybenzotriazole (HOBt, 0.46 g, 3 mmole) and DCC (0.41 g, 2 mmole) were dissolved in 50 ml of 4:1 DCM/DMF and stirred at 0°C for 1 hr. Aminomethyl-resin (1.34 g, 1 mmole available nitrogen (was suspended in the filtered reaction mixture (precipitated DCU removed) and shaken for 2 to 15 hours. The product, Boc-Phe-OCH_2-Pam-resin, was isolated by filtration and treated according to Table 1 (Steps 10-14) to give the desired free base, H-Phe-OCH_2-Pam-resin.

Example 12

H-MePhe-OCH_2-Pam-resin

Boc-MePhe-(4-oxymethylphenyl)acetic acid (from 1.82 g, 3 mmole, of its DCHA salt, Example 5) and HOBt (0.69 g, 4.5 mmole) in 40 ml of 1:3 DMF/DCM followed by DCC (0.62 g, 3 mmole) in 20 ml of DCM were added to aminomethyl-resin (1.34 g, 1 mmole available nitrogen) to give a suspension which was shaken for 2 to 15 hours. The desired product, Boc-MePhe-OCH_2-Pam-resin, was isolated by filtration, washed with 2-propanol and DCM, and treated according to Table 1 (Steps 10-14) to give the desired free base, H-MePhe-OCH_2-Pam-resin.

EXAMPLE 13

H-EtPhe-OCH_2-Pam-resin

Following the procedure of Example 12 but substituting Boc-EtPhe-(4-oxymethylphenyl)acetic acid (from 1.87 g, 3 mmole, of its DCHA salt, Example 6) for Boc-MePhe-(4-oxymethylphenyl)acetic acid, H-EtPhe-OCH_2-Pam-resin was obtained.

EXAMPLE 14

H-Phe(4-Cl)-OCH_2-Pam-resin

Following the procedure of Example 11 but substituting Boc-Phe(4-Cl)-(4-oxymethylphenyl)acetic acid (0.90 g, 2 mmole, Example 7) for Boc-Phe-(4-oxymethylphenyl)acetic acid, H-Phe(4-Cl)-OCH_2-Pam-resin was obtained.

EXAMPLE 15

H-Phe(4-NO_2)-OCH_2-Pam-resin

Boc-Phe(4-NO_2)-OH (1.39 g) was dissolved in 100 ml of 70% methanol (MeOH) and adjusted to pH 7 with the addition of N cesium bicarbonate. The solution was evaporated to dryness with the residue being evaporated three more times with added DMF. The resultant dried cesium salt of Boc-Phe(4-NO_2)-OH was dissolved in 60 ml of DMF and shaken with $BrCH_2$-Pam-resin (1 meq or Br) overnight. The desired product, Boc-Phe(4-NO_2)-OCH_2-Pam-resin, was isolated by filtration, washed with DCM, and treated according to Table 1 (Steps 10-14) to give the desired free base, H-Phe(4-NO_2)-OCH_2-Pam-resin.

EXAMPLE 16

H-Tyr(Me)-OCH_2-Pam-resin

Following the procedure of Example 11 but substituting Boc-Tyr(Me)-(4-oxymethylphenyl)acetic acid (0.87 g, 2 mmole, Example 8) for Boc-Phe-(4-oxymethylphenyl)acetic acid, H-Tyr(Me)-OCH_2-Pam-resin was obtained.

EXAMPLE 17

H-Tyr(tBu)-OCH_2-Pam-resin

Fmoc-Tyr(tBu)-(4-oxymethylphenyl)acetic acid (1.82 g, 3 mmole, Example 9), 1-hydroxybenzotriazole (HOBt, 0.69 g, 4.5 mmole), and DCC (0.62 g, 3 mmole) were dissolved in 50 ml of 4:1 DCM/DMF and stirred at 0°C for 1 hr. Aminomethylresin (1.34 g, 1 mmole available nitrogen) was suspended in the filtered reaction mixture (precipitated DCU removed) and shaken for 2 to 15 hours. The product, Fmoc-Tyr(tBu)-OCH_2-Pam-resin, was isolated by filtration and treated according to Table 1 (Steps 16-20) to give the desired free base, H-Tyr(tBu)-OCH_2-Pam-resin.

EXAMPLE 18

H-MeTyr(Me)-OCH_2-Pam-resin

Following the procedure of Example 15 but substituting Boc-MeTyr(Me)-OH (from 1.47 g of its DCHA salt) for Boc-Phe(N_2)-OH, H-MeTyr(Me)-OCH_2-Pam-resin was obtained.

EXAMPLE 19

H-DAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-DAsp-(OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step

30) to give 198 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.11 (2), Tyr 1.04 (1), Met 2.07 (2), Gly 1.08 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.35 (TLC herein refers to chromatography of the title compound on Merck silica gel thin layer plates in the solvent system chloroform-methanol-acetic acid-water, 6:3:1:1.

EXAMPLE 20

iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with iBuOCO-OSu (Example 1) according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 206 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.06 (1), Tyr 1.04 (1), Met 2.04 (2), Gly 1.06 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.56.

EXAMPLE 21

H-Asp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-Oh, Fmoc-DTyr-OH (Example 2), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Boc-Asp(OtBu)-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 241 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.14 92), Tyr 1.06 (1), Met 2.12 (2), Gly 0.95 (1), Phe 0.98 (1), and NH_3 1.30 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.32.

EXAMPLE 22

H-Asp-Tyr(SO_3H)-DMet-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-DMet-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-DMet-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 219 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.07 (2), Tyr 1.02 (1), Met 2.11 (2), Gly 0.95 (1), Phe 1.11 (1), and NH_3 1.31 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.37.

EXAMPLE 23

H-Asp-Tyr(SO_3H)-DAhx-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-DAhx-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-DAhx-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 248 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.11 (2), Tyr 1.03 (1), Ahx 0.97 (1), Gly 0.99 (1), Met 1.07 (1), and Phe 1.07 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.34.

EXAMPLE 24

H-Asp-Tyr(SO_3H)-Met-Sar-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Sar-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Sar-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 240 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.93 (2), Tyr 0.97 (1), Met 2.00 (2), Sar 1.03 (1), Phe 1.02 (1), and NH_3 1.54 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.32.

EXAMPLE 25

H-Asp-Tyr(SO_3H)-Met-Gly-MeTrp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-MeTrp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH ac cording to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-MeTrp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 310 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 1.02 (1), Met 1.95 (2), Gly 0.93 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.35.

EXAMPLE 26

H-Asp-Tyr(SO_3H)-Met-Gly-Nal-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Nal-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp-(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Gly-Nal-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step

30) to give 260 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.97 (2), Tyr 0.91 (1), Met 2.13 (2), Gly 1.09 (1), Nal 0.76 (1), and Phe 1.14 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.38.

EXAMPLE 27

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-DAsp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Gly-Trp-Met-DAsp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 121 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 0.98 (1), Met 2.05 (2), Gly 1.07 (1), and Phe 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.43.

EXAMPLE 28

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asn-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asn-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asn-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 1.03 (1), Met 1.93 (2), Gly 1.01 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.43.

EXAMPLE 29

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (free base of Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp-(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 243 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.14 (2), Tyr 1.02 (1), Met 2.04 (2), Gly 1.07 (1), and NH_3 1.87 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.37.

EXAMPLE 30

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MeTyr(Me)-N_2

H-MeTyr(Me)-OCH_2-Pam-resin (Example 18) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (free base of Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp-(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MeTyr(Me)-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.06 (2), Tyr 1.06 (1), Met 1.98 (2), and Gly 1.05 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.45.

EXAMPLE 31

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-NO_2)-NH_2

H-Phe(4-NO_2)-OCH_2-Pam-resin (Example 15) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe(4-NO_2)-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 128 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.98 (2), Tyr 1.05 (1), Met 1.92 (2), and Gly 1.05(1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.37.

EXAMPLE 32

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-Cl)-NH_2

H-Phe(4-Cl)-OCH_2-Pam-resin (Example 14) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe(4-Cl)-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 299 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.85 (2), Tyr 1.02 (1), Met 1.78 (2), and Gly 0.92 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.36.

EXAMPLE 33

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-NH_2)-NH_2

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-NO_2)-NH_2 (64 mg, Example 31) was dissolved in 85% acetic acid (20 ml) and treated with zinc dust (69 mg) with stirring. After 30 min, the filtered reaction mixture was evaporated to dryness and the residue was chromatographically purified on P-40 ODS-3 according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.92 (2), Tyr 0.98 (1), Met 1.87 (2), and Gly 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.35.

EXAMPLE 34

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Tyr(Me)-NH_2

H-Tyr(Me)-OCH_2-Pam-resin (Example 16) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Tyr(Me)-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 313 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.05 (2), Tyr 1.98 (2), Met 1.88 (2), and Gly 1.09 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.38.

EXAMPLE 35

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NHMe

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with methylamine) to give the title compound which was chromatographically purified on Trisacryl M DEAE and Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 196 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.99 (2), Tyr 1.00 (1), Met 1.98 (2) Gly, 1.00 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.47.

EXAMPLE 36

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NHEt

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ethylamine) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 180 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.99 (2), Tyr 0.84 (1), Met 2.03 (2), Gly 1.01 (1), Phe 0.97 (1), and NH3 1.14 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.49.

EXAMPLE 37

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-N($ME)_2$

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with dimethylamine) to give the title compound which was chromatographically purified on Trisacryl M

DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 1.01 (1), Met 1.97 (2,) Gly 0.99 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.35.

EXAMPLE 38

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-N($Et)_2$

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with diethylamine) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 102 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.16 (2), Tyr 1.03 (1), Met 1.92 (2), Gly 1.00 (1), and Phe 1.09 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.38.

EXAMPLE 39

H-βAsp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-DTyr-OH (Example 2) and Boc-βAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Boc-βAsp(OtBu)-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected according to Table 1 (Steps 10-15) and coupled with Fmoc-OSu (1.1 g) according to Table 1 (Steps 8-9) to give Fmoc-βAsp-DTyr-Met-Gly-Trp-Met-Asp-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to gave 121 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.94 (2), Tyr 1.02 (1), Met 1.91 (2), Gly 1.11 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.37.

EXAMPLE 40

H-DAsp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-DTyr-OH (Example 2) and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-DAsp-(OtBu)-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to gave 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.08 (2), Tyr 1.06 (1), Met 1.83 (2,) Gly 1.05 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.24.

EXAMPLE 41

Suc-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-DTyr-OH (Example 2) according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give H-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with succinic anhydride (0.6 g, 6 mmole, in DMF) according to Table 1 (Steps 8-9) to give Suc-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OC_2-Pam resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 290 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.08 (2), Tyr 1.04 (1), Met 1.70 (2), Gly 1.14 (1), and Phe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.51.

EXAMPLE 42

H-Asp-DTyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ile-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-Asp (OtBu)-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 370 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.05 (2), Tyr 1.00 (1), Ile 1.95 (2), Gly 1.00 (1), and Phe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.33.

EXAMPLE 43

H-Asp-Tyr(SO_3H)-Lys-Gly-Trp-Lys-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Lys-(Boc)-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-Asp (OtBu)-Tyr(tBu)-Lys(Boc)-Gly-Trp-Lys(Boc)-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 61 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.98 (2), Tyr 1.01 (1), Lys 2.00 (2) Gly 0.99 (1), and Phe 1.02 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.10.

EXAMPLE 44

Hpp(SO_3H)-Met-DAla-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-DAla-OH, and Fmoc-Met-OH, according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with Hpp-OSu according to Table 1 (Steps 8-9) to give Hpp-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatog-

raphically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 140 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02(1), Met 1.97 (2), Ala 0.98 (1), and Phe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.52.

EXAMPLE 45

Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2

H-Phe-OCH_2-Pam resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH, according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with succinic anhydride (0.6 g, 6 mmole, in DMF) according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 240 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.01 (1), Tyr 0.95 (1), Ahx 2.10 (2), Gly 1.06 (1), and Phe 0.88 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.36.

EXAMPLE 46

H-βAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Boc-βAsp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give Boc-βAsp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected according to Table 1 (Steps 10-15) and coupled with Fmoc-OSu 1.1 g) according to Table 1 (Steps 8-9) to give Fmoc-βAsp-Tyr-Met-Gly-Trp-Met-Asp-MePhe-OCH2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 72 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 1.05 (1), Met 1.85 (2), and Gly 1.10 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.33.

EXAMPLE 47

H-DAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-Oh, Fmoc-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-DAsp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromotographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 110 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.01 (2), Tyr 1.01 (1), Met 1.91 (2), and Gly 1.07 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.35.

EXAMPLE 48

For-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give Fmoc-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected according to Table 1 (Steps 10-20) and coupled with For-Tyr-OH according to Table 1 (Steps 5-7) to give For-Tyr-Met-Gly-Trp-Met-Asp-MePhe-OCH_2-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 78 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02 (1), Tyr 1.01 (1), Met 1.95 (2), and Gly 1.02 (1). Infrared absorption spectrum showed a strom peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.45.

EXAMPLE 49

iBuOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with iBuOCO-OSu (Example 1) according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 302 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.03 (1), Tyr 1.02 (1), Met 1.91 (2), and Gly 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.67.

EXAMPLE 50

H-Asp-DTyr(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmox-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-DTyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-Asp(OtBu)-DTyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Frisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 29 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 0.97 (1), Met 1.93 (2), and Gly 1.07 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.28.

EXAMPLE 51

Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give H-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with 3-(4-hydroxyphenyl)propionic acid N-hydroxysuccinimide ester (Hpp-OSu) according to Table 1 (Steps 8-9) to give Hpp-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then

Steps 26-29 with ammonia) to give the title compound which was chromatograhically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 170 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02 (1), Met 1.97 (2), and Gly 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.58.

EXAMPLE 52

Hpp(SO_3H)-Met-DAla-Trp-Met-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-DAla-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Met-DAla-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pan-resin which was coupled with Hpp-OSu according to Table 1 (Steps 8-9) to give Hpp-Met-DAla-Trp-Met-Asp-(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 84 mg of the ammonium salt of the title compound. Amino acid anaylsis following acid decomposition gave Asp 0.99 (1), Met 1.94 (2), and Ala 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.49.

EXAMLE 53

PrOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with PrOCO-OSu (mp 31-34°C, prepared according to the procedure of Example 1 except that propyl chloroformate was substituted for isobutyl chloroformate) according to Table 1 (Steps 8-9) to give PrOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15), Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 270 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.01 (1), Tyr 1.03 (1), Met 1.87 (2), Gly 1.02 (1), and Phe 1.06 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.45.

EXAMPLE 54

EtOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was seqentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with EtOCO-OSu (mp 52-54.5°C, prepared according to the procedure of Example 1 except that ethyl chloroformate was substituted for isobutyl chloroformate) according to Table 1 (Steps 8-9) to give EtOCO-Typ(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 300 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02 (1), Tyr 1.01 (1), Met 1.91 (2), Gly 1.02 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.44

EXAMPLE 55

MeOCO-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Typ-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with MeOCO-OSu (mp 87-89°C, prepared according to the procedure of Example 1 except that methyl chloroformate was substituted for isobutyl chloroformate) according to Table 1 (Steps 8-9) to give MeOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 270 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.04 (1), Tyr 1.05 (1), Met 1.82 (2), Gly 1.03 (1), and Phe 1.06 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.44.

EXAMPLE 56

H-βAsp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (Digestive Diseases, 15, 149-156 (1970)). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-βAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removel Steps 16-20) to provide Boc-βAsp(OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 283 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 0.94 (1), Met 2.08 (2), Gly 0.99 (1), and Phe 0.96 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.52.

EXAMPLE 57

H-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (U.S. Patents 3,839,315 and 3,705,140). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Boc-Tyr-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 240 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.05 (1), Tyr 1.04 (1), Met 2.10 (2), Gly 1.07 (1), and Phe 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.55.

EXAMPLE 58

Suc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (European Patent Application 0107860). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr-OH (Example 3) according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide H-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin

which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 246 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.03 (1), Tyr 0.95 (1), Met 2.08 (2), Gly 0.98 (1), and Phe 0.96 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.54.

EXAMPLE 59

Hpp(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (Int. J. Peptide Protein Res., 16, 402-411 (1980)). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp (OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide H-Met-Gly-Trp-Met-Asp (OtBu)-Phe-OCH_2-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Steps 8-9) to give Hpp-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 65 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.04 (1), Met 2.04 (2), Gly 0.95 (1), and Phe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.47.

EXAMPLE 60

H-Asp-Tyr(SO_3H)-Ahx-Gly-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Ahx-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 188 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.97 (2), Tyr 0.99 (1), Ahx 1.06 (1), Gly 1.07 (1), Met 0.93 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.57.

EXAMPLE 61

H-Asp-Tyr(So_3H)-Met-Gly-DTrp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-DTrp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-Gly-DTrp-Met-Asp-(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 314 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.07 (2), Tyr 1.01 (1), Met 2.01 (2), Gly 1.06 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.30.

EXAMPLE 62

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Ahx-Asp-Phe-NH_2

The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp (OtBu)-Tyr-Met-Gly-Trp-Ahx-Asp-(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 87 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.11 (2), Tyr 1.00 (1), Met 1.11 (1), Gly 1.02 (1), Ahx 1.04 (1), and Phe 0.99 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.23.

EXAMPLE 63

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-DPhe-NH_2

The title compound has previously been prepared (U.S. Patent 3,892,726). H-DPhe-OCH_2-Pam-resin {prepared in an analogous fashion as H-Phe-OCH_2-Pam-resin [Example 11, Boc-Phe-(4-oxymethylphenyl)-acetic acid replaced with Boc-DPhe-(4-oxymethylphenyl)acetic acid] from Boc-DPhe-(4-oxymethylphenyl)-acetic acid [Example 5, Boc-MePhe-OH replaced with Boc-DPhe-OH]} was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp (OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-DPhe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 141 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.97 (2), Tyr 0.98 (1), Met 2.03 (2), Gly 1.09 (1), and Phe 1.05 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.32.

EXAMPLE 64

H-Asp-Tyr(SO_3H)-MetO-Gly-Trp-MetO-Asp-Phe-NH_2

The title compound has previously been prepared (Nobel Symp., 16, (Front. Gastrointest. Horm. Res.), 41-56 (1973)). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-MetO-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-MetO-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp-(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-MetO-Gly-Trp-MetO-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 162 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.09 (2), Tyr 1.00 (1), MetO 1.83 (2), Gly 1.08 (1), and Phe 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.14.

EXAMPLE 65

H-Asp-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2

The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH_2-Pam-resin (Example 11) was sequentialy coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Bos-Asp (OtBu)-Tyr-Ahx-Gly-Trp-Ahx-Asp-(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 94 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.18 (2), Tyr 1.06 (1), Ahx 2.01 (2), Gly 1.10 (1), and Phe 0.94 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.32.

EXAMPLE 66

H-Asp-Tyr(SO_3H)-Leu-Gly-Trp-Leu-Asp-Phe-NH_2

The title compound has previously been prepared (Digestive Diseases, 15, 149-156 (1970)). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Leu-Gly-Trp-Leu-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 150 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 0.99 (1), Leu 2.02 (2), Gly 0.98 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.35.

EXAMPLE 67

For-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (U.S. Patent 3,705,140). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide H-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected according to Table 1 (Steps 10-15) to provide H-Met-Gly-Trp-Met-Asp-Phe-OCH_2-Pam-resin which was coupled with For-Tyr-OH according to Table 1 (Steps 3-4) to give For-Tyr-Met-Gly-Trp-Met-Asp-Phe-OCH_2-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.00 (1), Met 1.98 (2), Gly 1.07 (1), and Phe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.37.

EXAMPLE 68

H-Asp-Tyr(SO_3H)-MetO-Gly-Trp-Met-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OTBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-MetO-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-MetO-Gly-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleav-

ed from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographicaaly purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 230 mg of the ammonium salt of the title compound. Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.26.

EXAMPLE 69

H-Asp-Tyr(SO_3H)-Met-DAla-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (Peptides 1984, 383-385 (1984)). H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-DAla-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp (OtBu)-Tyr-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and Amberlite XAD-2, sequentially, according to Table 1 (Step 30) to give 140 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.93 (2), Tyr 0.94 (1), Met 1.81 (2), Ala 0.97 (1), and Phe 0.95 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.34.

EXAMPLE 70

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-DMet-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-DMet-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Gly-Trp-DMet-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 368 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.90 (2), Tyr 0.99 (1), Met 1.97 (2), Gly 0.92 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.31.

EXAMPLE 71

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-MetO-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-MetO-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Gly-Trp-MetO-Asp(OtBu)-Phe-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatograph-ically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 126 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 0.96 (1), Met 0.98 (1), Gly 1.10 (1), MetO 0.97 (1), and Phe 0.96 (1). infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.29.

EXAMPLE 72

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe(4-Me)-NH_2

H-Phe(4-Me)-OCH_2-Pam-resin {prepared in an analogous fashion as H-Phe-OCH_2-Pam-resin [Example 11, Boc-Phe-(4-oxymethylphenyl)-acetic acid replaced with Boc-Phe(4-Me)-(4-oxymethylphenyl)acetic acid] from Boc-Phe(4-Me)-(4-oxymethylphenyl) acetic acid [Example 5, Boc-MePhe-OH replaced with Boc-Phe(4-Me)-OH]} was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe(4-Me)-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and p-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 420 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 1.00 (1), Met 1.91 (2), Gly 1.06 (1), and Phe (4-Me) 1.12 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.44.

EXAMPLE 73

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Tyr-NH_2

H-Tyr(tBu)-OCH_2-Pam-resin {prepared in an analogous fashion as H-Phe-OCH_2-Pam-resin [Example 11, Boc-Phe-(4-oxymethylphenyl)-acetic acid replaced with Fmoc-Tyr(tBu)-(4-oxymethylphenyl)-acetic acid] from Boc-Tyr(tBu)-(4-oxymethylphenyl) acetic acid [Example 5, Boc-MePhe-OH replaced with Fmoc-Tyr(tBu)-OH]} was sequentially coupled with Fmox-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-Gly-Trp-Met-Asp-(OtBu)-Tyr (tBu)-OCH_2-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 42 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.09 (2), Tyr 2.16 (2), Met 1.66 (2), and Gly 1.09 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.29.

EXAMPLE 74

Hpp(SO_3H)-Met-Gly-Trp-Pro-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Met-Gly-Trp-Pro-Asp-(OtBu)-Phe-OCH_2-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Steps 8-9) to give Hpp-Met-Gly-Trp-Pro-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 70 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Met 1.01 (1), Gly 1.04 (1), Pro 0.97 (1), Asp (1.04) (1), and Phe 0.94 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.51.

EXAMPLE 75

Hpp(SO_3H)-Pro-Gly-Trp-Pro-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Pro-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Pro-Gly-Trp-Pro-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Steps 8-9) to give Hpp-Pro-Gly-Trp-Pro-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 480 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Pro 2.02 (2), Gly 1.05 (1), Asp 1.00 (1), and Phe 0.93 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.44.

EXAMPLE 76

Suc-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ahx-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ahx-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr (tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.97 (1), Gly 1.00 (1), and Asp 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.46.

EXAMPLE 77

iBuOCO-Tyr(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ahx-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ahx-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr (tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp-(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 290 mg of the ammonium salt of the title compound. Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.54.

EXAMPLE 78

Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ahx-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ahx-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ahx-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Steps 8-9) to give Hpp-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2,

Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 91 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ahx 1.92 (2), Gly 1.03 (1), and Asp 1.05 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.40.

EXAMPLE 79

iBuOCO-Tyr(So_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 800 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.90 (1), Ahx 1.84 (2), Gly 1.12(1), Asp 1.12 (1), and Phe 0.92 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.58.

EXAMPLE 80

Hpp(SO_3H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ahx-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ahx-Gly-Trp-Ahx-Asp-(OtBu)-Phe-OCH_2-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Step 8-9) to give Hpp-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 450 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ahx 1.92 (2), Gly 1.01(1), Asp 1.10(1), and Phe 0.97(1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.47.

EXAMPLE 81

Suc-Tyr(SO_3H)-Met-DAla-Trp-Met-Asp-Phe-NH_2

The title compound has previously been prepared (Peptides 1984, 373-378 (1984)). H-Phe-OCH_2-Pam-resin (Exmaple 11) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-DAla-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide H-Tyr (tBu)-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 110 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.02 (1), Met 1.93 (2), Ala 1.00 (1), Asp 1.05 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.38.

EXAMPLE 82

For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected according to Table 1 (Steps 10-15) to provide H-Ile-Gly-Trp-Ile-Asp-Phe-OCH_2-Pam-resin which was coupled with For-Tyr-OH according to Table 1 (Steps 3-4) to give For-Tyr-Ile-Gly-Trp-Ile-Asp-Phe-OCH_2-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 200 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.98 (1), Ile 1.94 (2), Gly 1.00 (1), Asp 1.09 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.59.

EXAMPLE 83

Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-Oh, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr-(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 300 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.00 (1), Ile 2.07 (2), Gly 0.97 (1), Asp 0.97 (1), and Phe 0.99 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.42.

EXAMPLE 84

iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr-(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-PheOCH$_2$-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 390 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.07 (1), Ile 2.21 (2), Gly 1.04 (1), Asp 1.00 (1), and Phe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.61.

EXAMPLE 86

Hpp (SO_3H)-Ile-Gly-Trp-Ile-Asp-Phe-NH_2

H-Phe-OCH_2-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to povide H-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH_2-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Step 8-9) to give Hpp-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the

resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 280 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ile 2.04 (2), Gly 0.99 (1), Asp 0.98 (1), and Phe 0.99 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.52.

EXAMPLE 87

Suc-Tyr(So_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2

The title compound has previously been prepared (N. Yanaihara, et al., in Peptides 1984: Proceedings of the 18th European Peptide Symposium, U. Ragnarsson, Ed., Almqvist and Wiksell International, Publisher, Stockholm, Sweden, 1985, pp 373-378). H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10) Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 170 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.02 (1), Met 1.94 (2), Gly 1.09 (1), Asp 1.00 (1), and MePhe 1.02 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC Rf 0.58.

EXAMPLE 88

H-DAsp-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-Oh (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBus)-OH, and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-DAsp(OtBu)-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.96 (2), Tyr 0.97 (1), Ile 1.92 (2), Gly 0.98 (1), and MePhe 1.18 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC Rf 0.36.

EXAMPLE 89

For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected according to Table 1 (Steps 10-15) to provide H-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin which was coupled with For-Tyr-OH according to Table 1 (coupling Steps 3-4) to give For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 LDS-3, sequentially, according to Table 1 (Step 30) to give 90 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.04 (1), Ile 1.95 (2), Gly 0.97 (1), Asp 1.01 (1), and MePhe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.60.

EXAMPLE 90

Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 130 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.94 (1), Ile 1.92 (2), Gly 1.04 (1), Asp 1.09 (1), and MePhe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.37.

EXAMPLE 91

iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu-Ile-Gly-Trp-Ile-Asp-(OtBu)-MePhe-OCH_2-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 50 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.00 (1), Ile 1.92 (2), Gly 1.02 (1), Asp 1.03 (1), and MePhe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.74.

EXAMPLE 92

H-Asp-DTyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-DTyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-DTyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH_2-Pam-Resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 200 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.08 (2), Tyr 1.01 (1), Ile 1.94 (2), Gly 0.98 (1), and MePhe 0.99 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.38.

EXAMPLE 93

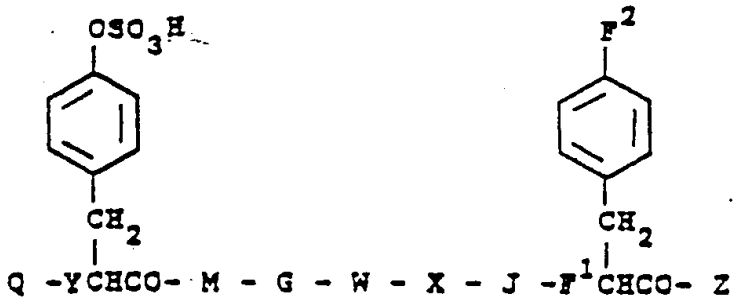
Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2

H-MePhe-OCH_2-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH_2-Pam-resin which was coupled with Hpp-OSu according to Table 1 (coupling

Steps 8-9) to give Hpp-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH$_2$-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 70 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ile 1.94 (2), Gly 1.01 (1), Asp 1.02 (1), and MePhe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm^{-1}. TLC R_f 0.63.

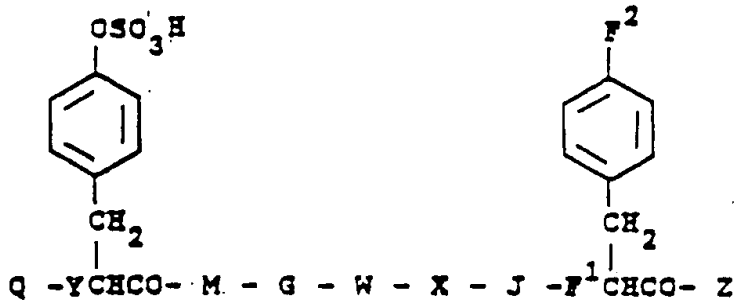
Claims

1. A peptide of the formula



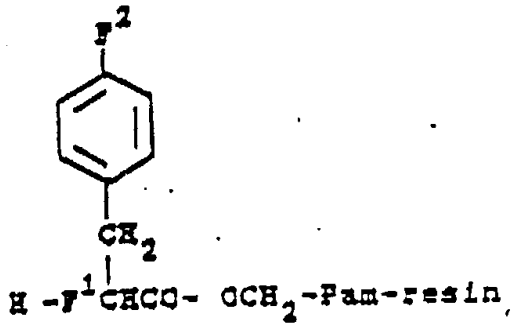
wherein
Q is H-βAsp, For, Suc, desQ, or R^1R^2CHOCO,
Y is H or (S)-NH,
M is Met, Ahx, Leu, or Ile,
G is Gly,
W is Trp,
X is Met, Ahx, Leu, or Ile,
J is Asp,
F^1 is (S)-NH or (S)-R^4N,
F^2 is H, NO_2, R^5, or OR^6,
Z is NH_2
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, R^5, and R^6 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that
(1) Q is desQ when Y is H,
(2) F^2 is not H if, in the same peptide, Q is H-BAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or Leu, X is either Met, Ahx, or Leu, and F^1 is (S)-NH,
(3) F^2 is not H if, in the same peptide, Y is H, M is Met, X is Met, and F^1 is (S)-NH,
(4) F^2 is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F^1 is (S)-R^4N.

2. A peptide of Claim 1 in which Q is iBuOCO.
3. A peptide of Claim 1 in which Q is Suc.
4. A peptide of Claim 1 in which Q is For.
5. A peptide of the formula

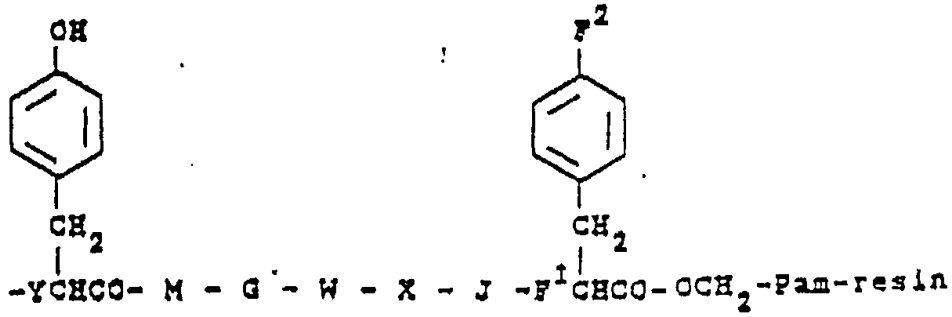


wherein
Q is H, H-Asp-H-βAsp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R^1R^2CHOCO,
Y is (R)-NH,
M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, MeIle or Lys,
G is Gly, DAla, Pro or Sar,
W is Trp, MeTrp or Nal,
X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, MeIle, Pro, or Lys,
J is Asp, DAsp, MeAsp, or Asn,
F^1 is (S)-NH, (S)-R^4N, or (R)-R^4N,
F^2 is H, Cl, I, Br, F, NO_2, NH_2, R^5, or OR^6,
Z is NH_2, NHR^7 or NR^7R^8,
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, and R^5 are lower alkyl,
R^6 is H or lower alkyl, and
R^7 and R^8 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that
(1) F^2 is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F^1 is (S)-NH and Z is NH_2.

6. A process for preparing a compound of Claim 1 or 5 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligo peptide to an amino-acyl resin of the formula

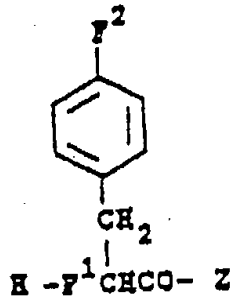


to afford a protected peptidyl resin of the formula

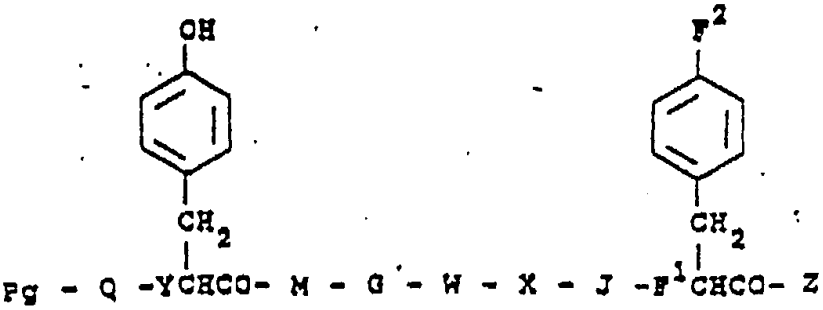


which is sulfated, deprotected, and treated with a methanolic amine.

7. The process for preparing a compound of Claim 1 or 5 by the solution phase method of sequential addition (followed by deprotection after each addition) fo the requisite protected amino acid or protected oligo peptide to an amino-acyl amide of the formula



to afford a protected peptide amide of the formula



which is sulfated and deprotected.

8. The peptide H-DAsp-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2.

9. The peptide For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2.

10. The peptide Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2.

11. The peptide iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Asp-MePhe-NH_2.

12. The peptide Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2.

13. The peptide of claim 5 that is H-Asp-DTyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2.

14. A process for preparing the compound of claim 8 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin H-DAsp-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

15. A process for preparing the compound of claim 9 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin For-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

16. A process for preparing the compound of claim 10 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin Suc-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

17. A process for preparing the compound of claim 11 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin iBuOCO-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

18. A process for preparing the compound of claim 12 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin Hpp-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

19. A process for preparing the compound of claim 13 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected aligopeptide to the amino-acryl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin H-Asp-DTyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

20. A process for preparing the compound of claim 8 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protect ed amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-DAsp-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

21. A process for preapring the compound of claim 9 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and dprotected.

22. A process for preparing the compound of claim 10 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-Suc-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

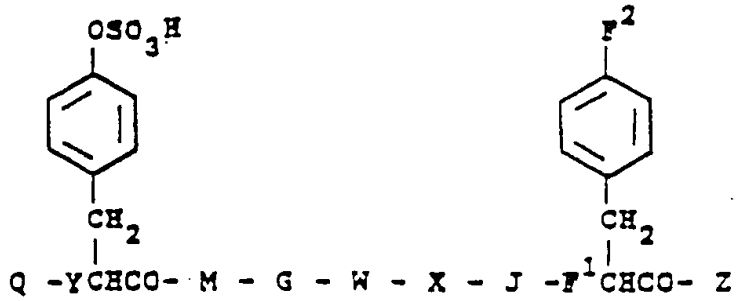
23. A process for preparing the compound of claim 11 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-iBuOCO-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

24. A process for preparing the compound of claim 12 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-Hpp-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-NH_2, which is sulfated and deprotected.

25. A process for preparing the compound of claim 13 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-Asp-DTyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

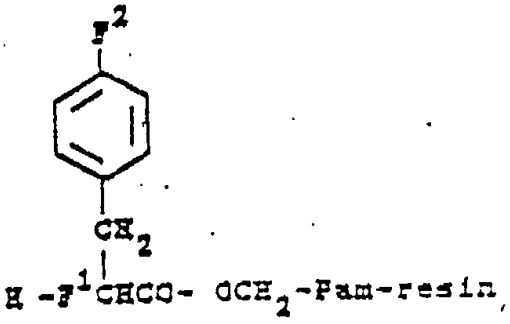
Claims for the following contracting States: GR and ES.

1. A process for preparing a peptide of the formula

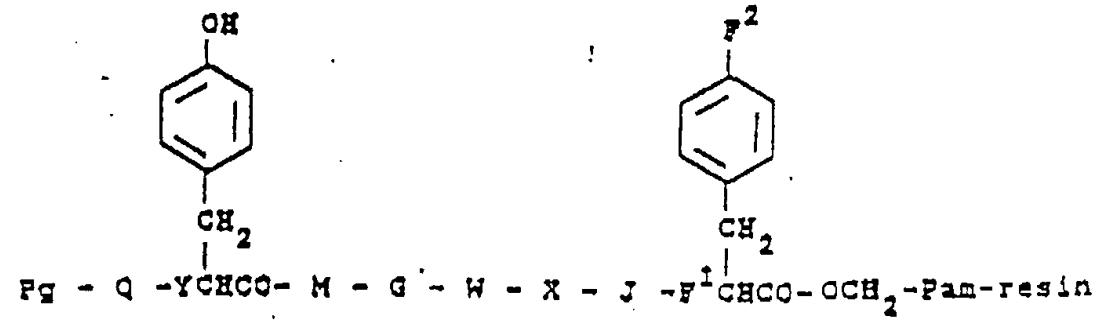


wherein

Q is H-βAsp, For, Suc, desQ, or R^1R^2CHOCO,
Y is H or (S)-NH,
M is Met, Ahx, Leu, or Ile,
G is Gly,
W is Trp,
X is Met, Ahx, Leu, or Ile,
J is Asp,
F^1 is (S)-NH or (S)-R^4N,
F^2 is H, NO_2, R^5, or OR^6,
Z is NH_2
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, R^5, and R^6 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that
(1) Q is desQ when Y is H,
(2) F^2 is not H if, in the same peptide, Q is H-BAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or Leu, X is either Met, Ahx, or Leu, and F^1 is (S)-NH,
(3) F^2 is not H if, in the same peptide, Y is H, M is Met, X is Met, and F^1 is (S)-NH,
(4) F^2 is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F^1 is (S)-R^4N, by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligo peptide to an amino-acyl resin of the formula

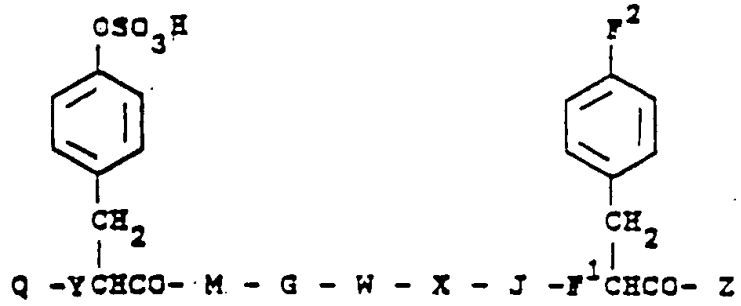


to afford a protected peptidyl resin of the formula

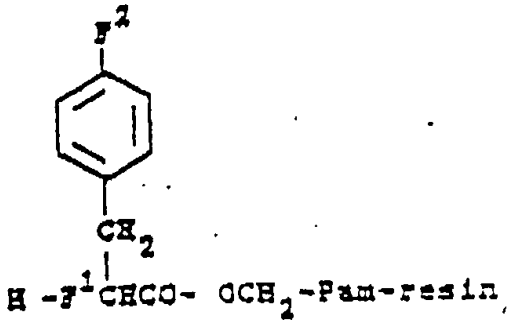


which is sulfated, deproteced, and treated with a methanolic amine.

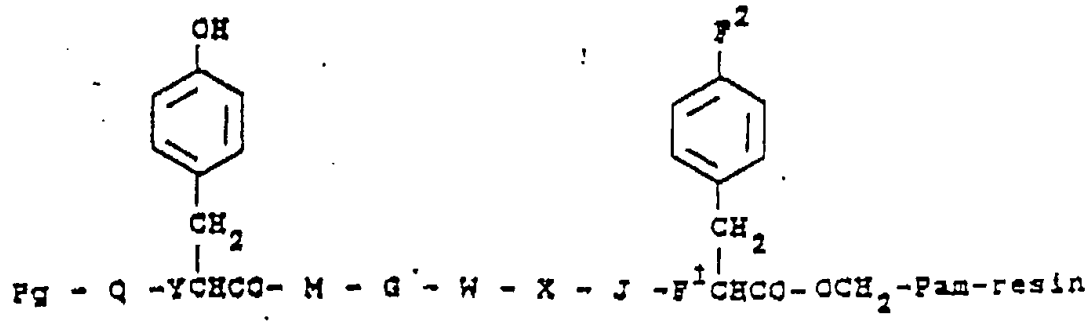
2. A process for preparing a peptide of the formula



wherein
Q is H, H-Asp, H-βAsp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R^1R^2CHOCO,
Y is (R)-NH,
N is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, MeIle or Lys,
G is Gly, DAla, Pro or Sar,
W is Trp, MeTrp or Nal,
X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, MeIle, Pro, or Lys,
J is Asp, DAsp, MeAsp, or Asn,
F^1 is (S)-NH, (S)-R^4N, or (R)-R^4N,
F^2 is H, Cl, I, Br, F, NO_2, NH_2, R^5, or OR^6,
Z is NH_2, NHR^7 or NR^7R^8,
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, and R^5 are lower alkyl,
R^6 is H or lower alkyl, and
R^7 and R^8 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that
(1) F^2 is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F^1 is (S)-NH and Z is NH_2, by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligo peptide to an amino-acyl resin of the formula

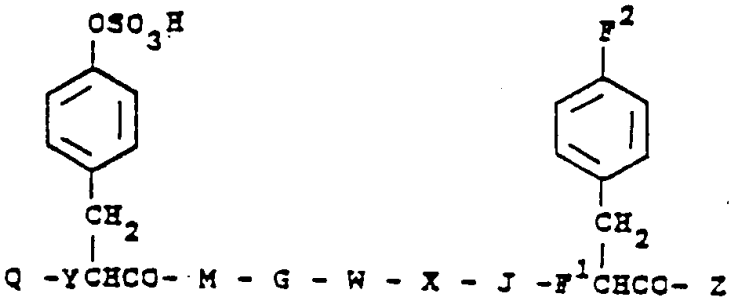


to afford a protected peptidyl resin of the formula

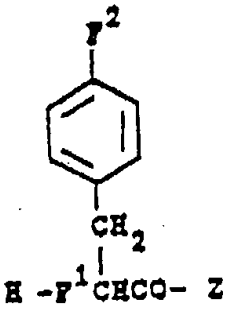


which is sulfated, deprotected, and treated with a methanolic amine.

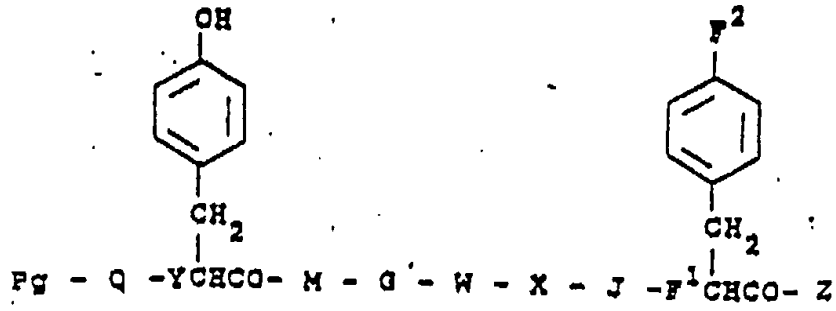
3. A process for preparing a peptide of the formula



wherein
Q is H-βAsp, For, Suc, desQ, or R^1R^2CHOCO,
Y is H or (S)-NH,
M is Met, Ahx, Leu, or Ile,
G is Gly,
W is Trp,
X is Met, Ahx, Leu, or Ile,
J is Asp,
F^1 is (S)-NH or (S)-R^4N,
F^2 is H, NO_2, R^5, or OR^6,
Z is NH_2
R^1 and R^2 are independently H or lower alkyl,
R^3, R^4, R^5, and R^6 are lower alkyl,
and pharmaceutically acceptable salts thereof,
provided that
(1) Q is desQ when Y is H,
(2) F^2 is not H if, in the same peptide, Q is H-BAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or Leu, X is either Met, Ahx, or Leu, and F^1 is (S)-NH,
(3) F^2 is not H if, in the same peptide, Y is H, M is Met, X is Met, and F^1 is (S)-NH,
(4) F^2 is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F^1 is (S)-R^4N, by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligo peptide to an amino-acyl amide of the formula

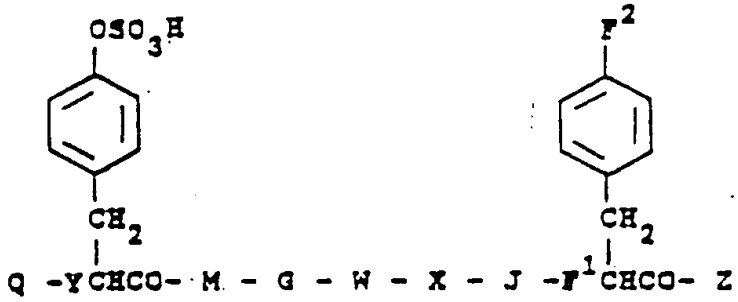


to afford a protected peptide amide of the formula



which is sulfated and deprotected.

4. A process for preparing a peptide of the formula



wherein

Q is H, H-Asp, H-βAsp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R^1R^2CHOCO,

Y is (R)-NH,

M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, MeIle or Lys,

G is Gly, DAla, Pro or Sar,

W is Trp, MeTrp or Nal,

X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, MeIle, Pro, or Lys,

J is Asp, DAsp, MeAsp, or Asn,

F^1 is (S)-NH, (S)-R^4N, or (R)-R^4N,

F^2 is H, Cl, I, Br, F, NO_2, NH_2, R^5, or OR^5,

Z is NH_2, NHR^7 or NR^7R^8,

R^1 and R^2 are independently H or lower alkyl,

R^3, R^4, and R^5 are lower alkyl,

R^6 is H or lower alkyl, and

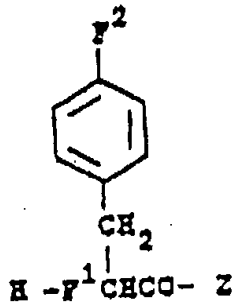
R^7 and R^8 are lower alkyl,

and pharmaceutically acceptable salts thereof,

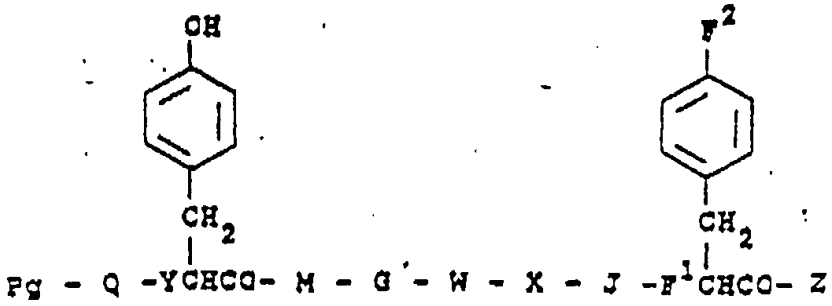
provided that

(1) F^2 is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F^1 is (S)-NH and Z is

NH_2, by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligo peptide to an amino-acyl amide of the formula



to afford a protected peptide amide of the formula



which is sulfated and deprotected.

5. A process for preparing the compound H-DAsp-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin H-DAsp-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

6. A process for preparing the compound For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

7. A process for preparing the compound Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin Suc-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

8. A process for preparing the compound iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected aligopeptide to the amino-acryl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin iBuOCO-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

9. A process for preparing the compound Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin Hpp-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

10. A process for preparing the compound H-Asp-DTyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protection amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH_2-Pam-resin to afford the peptidyl resin H-Asp-DTyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-Pam-resin, which is sulfated and treated with a methanolic amine.

11. A process for preparing the compound H-DAsp-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solution phase method of sequential addition (followed by deprotection after each addition of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-DAsp-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

12. A process for preparing the compound For-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

13. A process for preparing the compound Suc-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-Suc-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

14. A process for preparing the compound iBuOCO-Tyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-iBuOCO-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

15. A process for preparing the compound Hpp(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-Hpp-Ile-Gly-Trp-Ile-Asp-MePhe-OCH_2-NH_2, which is sulfated and deprotected.

16. A process for preparing the compound H-Asp-DTyr(SO_3H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH_2 to afford the protected peptide amide Pg-Asp-DTyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH_2, which is sulfated and deprotected.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 268 297**
A3

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 87117096.5

(51) Int. Cl.5: **C07K 7/30**

(22) Date of filing: **19.11.87**

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27.05.87 US 54554

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AT BE CH DE ES FR GB GR IT LI LU NL SE

(88) Date of deferred publication of the search report:
29.08.90 Bulletin 90/35

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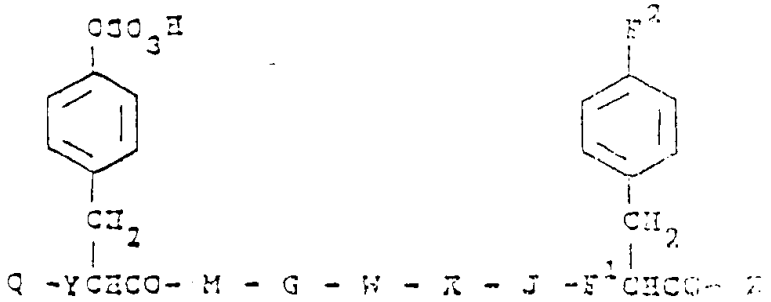
(54) Peptides with sulfate ester groups.

(57) Novel peptides having sulfate ester groups and containing 6 to 9 amino acids of the formula



possess feeding inhibition properties and capable of stimulating the contraction of the gallbladder. Also methods of treating and preventing obesity in which these novel peptides or other specified peptides can be used.

EP 0 268 297 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application number
EP 87 11 7096

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl 4)
X	CHEMICAL ABSTRACTS, vol. 104, no. 21, May 26, 1986, page 511, abstract no. 184817b, Columbus, Ohio, US; & JP-A-60 197 699 (FUJI CHEMICALS INDUSTRIAL CO. LTD.) 07-10-1985 * Abstract * --	1,5,7	C 07 K 7/30
Y	CHEMICAL ABSTRACTS, vol. 102, no. 15, April 15, 1985, pages 671, abstract no. 132473r, Columbus, Ohio, US; & EP-A-0 124 420 (SANOFI; CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIC) 07-11-1984 * Abstract * --	1-7, 9-12, 15-18, 21-24	TECHNICAL FIELDS SEARCHED (Int Cl 4) C 07 K
Y	CHEMICAL ABSTRACTS, vol. 89, no. 13, September 25, 1978, page 1006, abstract no. 110419p, Columbus, Ohio, US; & DD-A-2 751 026 (AKADEMIE DER WISSENSCHAFTEN DER DDR) 01-06-1978 * Abstract * --	1-7, 9-12, 15-18, 21-24	

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Place of search	Date of completion of the search	Examiner
THE HAGUE	12-02-1990	MASTURZO

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO Form 1503 03.82

European Patent Office

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

X LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

See sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims: 1-7(partially),9-12,15-18,21-24(completely)

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl 4)
Y	CHEMICAL ABSTRACTS, vol. 97, no. 25, December 20, 1982, page 905, abstract no. 216686d, Columbus, Ohio, US; M. BODANSZKY et al.: "Application of the 9-fluorenylmethyloxy-carbonyl (FMOC) group in peptide synthesis in solution" & PEPT., PROC. EUR. PEPT. SYMP., 16th 1980 (Pub. 1981), 93-8 * Abstract *	1-7, 9-12, 15-18, 21-24	
Y	US-A-4 490 364 (RIVIER et al.) * The whole document *	1-7, 9-12, 15-18, 21-24	TECHNICAL FIELDS SEARCHED (Int Cl 4)
Y	US-A-4 351 829 (ZETLER et al.) * The whole document, especially column 1, lines 1-20 *	1-7, 9-12, 15-18, 21-24	
Y	EP-A-0 107 860 (AMANO) * The whole document, especially page 46, claim 1 *	1-7, 9-12, 15-18, 21-24	

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Place of search	Date of completion of the search	Examiner

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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Application number
EP 87 11 7096

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	EP-A-0 161 468 (PENNWALT) * The whole document * ----	1-7, 9-12, 15-18, 21-24	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)

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